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## Research Article

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# Visible-Light-Active Photosensitized TiO<sub>2</sub> Nanomaterials for Antimicrobial Photodynamic Inactivation: Interfacial Photochemistry and Iodide Potentiation

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## Abstract

Photodynamic inactivation (PDI) can exploit molecular photosensitizer photochemistry and semiconductor-mediated redox processes, while biological activity depends on how these pathways operate at the material–microbe interface. Colloidal nanocrystalline TiO<sub>2</sub> (qTiO<sub>2</sub>) was surface-sensitized with 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (TCPP), 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (THPP), its zinc(II) complex (ZnTHPP), or hypericin (Hyp). The free porphyrins were substantial singlet-oxygen sensitizers, with  $\Phi_{\Delta}$  values of 0.7, 0.5, and 0.4 for TCPP, THPP, and ZnTHPP, respectively. Association with qTiO<sub>2</sub> quenched porphyrin fluorescence and extended photocurrent generation into the visible region, supporting photoinduced electron transfer at the sensitizer–semiconductor interface. Measurements with APF, DHE, and SOSG showed that the sensitized materials generated both oxygen-centred radicals and singlet oxygen, providing access to Type I and Type II photochemistry under visible-light excitation. THPP@qTiO<sub>2</sub> showed strong contributions from both routes. Photochemical activity did not directly predict antibacterial efficacy. Under  $420 \pm 20$  nm irradiation ( $10 \text{ J cm}^{-2}$ ), THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub> showed strong photodynamic activity against *Staphylococcus aureus*, reducing viable counts by approximately 5 and >4 log units, respectively, whereas TCPP@qTiO<sub>2</sub> was only weakly active. *Klebsiella pneumoniae* was unaffected without potentiation. Addition of 100 mM KI further enhanced *S. aureus* killing, with TCPP@qTiO<sub>2</sub>, THPP@qTiO<sub>2</sub>, and ZnTHPP@qTiO<sub>2</sub> reducing viable counts to the detection limit. KI also rendered TCPP@qTiO<sub>2</sub> highly active against *K. pneumoniae*, reducing viability to the detection limit (>5-log reduction). Hyp@qTiO<sub>2</sub> aggregated under bacterial assay conditions but showed increased antimicrobial activity after KI addition. Photodynamic efficacy therefore reflects the combined influence of molecular and semiconductor photochemistry, interfacial and colloidal properties, bacterial-envelope accessibility, and iodide-mediated secondary chemistry.

**Keywords:** photodynamic inactivation; photosensitized TiO<sub>2</sub>; porphyrins; reactive oxygen species (ROS); nanomaterials; interfacial electron transfer; potassium iodide (KI)

## Keypoints:

- qTiO<sub>2</sub> sensitization reshapes the balance between radical- and singlet-oxygen-associated photochemistry.
- Cell-free ROS generation does not predict antibacterial efficacy of sensitized qTiO<sub>2</sub>.
- KI potentiation renders TCPP@qTiO<sub>2</sub> highly active against Gram-negative *K. pneumoniae*.

## 1. Introduction

Antimicrobial resistance (AMR) remains a major challenge in medicine and biotechnology, with multidrug-resistant (MDR) pathogens progressively limiting the effectiveness of conventional antimicrobial treatment

(Lescho and Laguio-Vila 2019; Murray C 2022). Nanoparticle-based systems are being investigated as complementary antimicrobial platforms, including functional nanomaterials designed for direct antimicrobial, antibiofilm, or light-activated activity (Abdallah et al. 2026; Pelgrift and Friedman 2013; Salma Banu. A 2026; Ziental et al. 2024a).

Photodynamic inactivation (PDI) offers an antimicrobial mode of action fundamentally different from that of conventional antibiotics. Following absorption of light by a photosensitizer (PS) and population of its triplet excited state, electron- or energy-transfer reactions generate reactive oxygen species (ROS) capable of oxidizing multiple cellular constituents rather than inhibiting a single molecular target (Cieplik et al. 2018; Kawczyk-Krupka et al. 2018; Pires et al. 2025; Pucelik et al. 2024; Pucelik and Dąbrowski 2022). Type I photochemistry involves electron or hydrogen-atom transfer to surrounding substrates or molecular oxygen, leading to oxygen-centred radicals such as superoxide ( $O_2^{\cdot-}$ ) and hydroxyl radicals ( $HO^{\cdot}$ ), whereas Type II energy transfer to ground-state molecular oxygen produces singlet oxygen ( $^1O_2$ ) (Baptista et al. 2017; Dąbrowski 2017). Both pathways can contribute to microbial killing, and their relative importance depends on the sensitizer structure and its local physicochemical environment (Barczyk et al. 2026). Singlet oxygen is particularly relevant to PDI because microorganisms possess relatively limited enzymatic protection against  $^1O_2$ -mediated oxidation compared with their defense systems against several radical ROS, while the simultaneous damage of lipids, proteins, and nucleic acids reduces the likelihood of classical target-specific resistance (Barczyk et al. 2026; Hamblin and Hasan 2004). The broader antimicrobial potential of visible-light-based approaches is also illustrated by the inactivation of a clinical *Mycobacterium tuberculosis* isolate using broad-spectrum irradiation in the 400–420 nm range (D'Agostini et al. 2026).

In nanomaterial-assisted PDI, colloidal stability, surface properties, and photosensitizer organization influence both photochemical activity and interaction with microbial cells. Many organic photosensitizers possess favorable intrinsic excited-state properties but are limited by poor aqueous solubility, aggregation-induced deactivation in physiological media, or insufficient photostability (Mfouo-Tynga et al. 2021). Association with inorganic nanomaterials can alter sensitizer organization while introducing interfacial electron-transfer processes that are unavailable to the molecular photosensitizer in homogeneous solution (Dąbrowski et al. 2015; Ribeiro et al. 2026; Sułek et al. 2020). Nanocrystalline titanium(IV) oxide ( $TiO_2$ ) is particularly attractive in this context because of its chemical and photochemical stability, established photocatalytic properties, and broad use in biomedical and environmental photocatalysis (Etacheri et al. 2015; Fujishima and Honda 1972; Serpa et al. 2008; Ziental et al. 2020). Direct excitation of anatase form of  $TiO_2$ , however, requires predominantly UV light because of its optical band gap of approximately 3.2 eV (Macwan et al. 2011). This restricts its biomedical utility, as UV light penetrates tissues poorly and may damage endogenous biomolecules and mammalian cells (Matsumura and Ananthaswamy 2004).

Surface sensitization with visible-light-absorbing chromophores provides a means of overcoming this spectral limitation. The approach has been demonstrated for several classes of organic modifiers, including porphyrins, metalloporphyrins, and anthraquinone derivatives (Abdallah et al. 2026; Barczyk et al. 2026; Sułek et al. 2019a; Sułek et al. 2019b; Trochowski et al. 2023). Porphyrins are particularly suited to this purpose because peripheral substitution and metal coordination allow their absorption, redox properties, aggregation behavior, and excited-state photochemistry to be adjusted over a broad range (Monteiro et al. 2019; Nikoloudakis et al. 2022; Ziental et al. 2024b). Importantly, porphyrins are intrinsically efficient triplet-state photosensitizers and can generate  $^1O_2$  through energy transfer to molecular oxygen, whereas  $TiO_2$  photocatalysis is primarily associated with charge-carrier-mediated redox chemistry and the formation of oxygen-centred radicals. Immobilization of a porphyrin at the semiconductor surface can therefore combine these complementary modes of ROS generation within a single visible-light-responsive material. Following chromophore excitation, electron transfer to  $TiO_2$  provides an additional route for charge separation and subsequent oxygen reduction, while the molecular sensitizer can retain the ability to populate the Type II pathway (Malec et al. 2023; Serpa et al. 2008). The balance between these processes depends on energetic alignment and electronic coupling, as well as on the distance, orientation, and anchoring mode of the chromophore at the semiconductor surface (Malec et al. 2023; Monteiro et al. 2019). Thus, visible-light sensitization of  $TiO_2$  can provide simultaneous access to molecular energy transfer and semiconductor-mediated electron-transfer chemistry rather than simply reproducing the photochemistry of either component alone.

Hypericin is a structurally distinct photosensitizer with strong visible-light absorption and established photodynamic activity (Agostinis et al. 2002). Its extended aromatic structure is associated with low aqueous solubility and a pronounced tendency to aggregate in polar media, which can substantially alter its photophysical and photochemical behavior. Incorporation of hydrophobic photosensitizers into nanostructured or supramolecular systems provides one strategy for improving their dispersion and maintaining photoactivity in aqueous environments (Pucelik et al. 2016a). Hypericin was therefore included alongside the porphyrin derivatives as a chemically distinct comparator to examine how sensitizer structure and interaction with qTiO<sub>2</sub> affect colloidal behavior and photochemical performance.

The biological consequences of ROS generation depend strongly on the architecture of the microbial envelope. Gram-negative bacteria are generally more difficult to photoinactivate than Gram-positive species because their outer membrane presents an additional permeability barrier and restricts access of many photosensitizers to susceptible cellular structures (Barczyk et al. 2026). This limitation is especially important for nanostructured photosensitizing systems, because short-lived ROS generated at or near the particle surface can exert efficient antimicrobial action only when oxidative chemistry occurs sufficiently close to the microbial target. Consequently, high ROS-generating capacity in a cell-free system does not necessarily translate into effective PDI of Gram-negative bacteria. Potassium iodide (KI) offers a means of extending the consequences of the primary photochemical reaction. Oxidation of I<sup>-</sup> by photogenerated ROS yields transient iodine radicals and subsequently longer-lived oxidized iodine products, including molecular iodine and triiodide, thereby extending antimicrobial oxidation beyond the short diffusion range of the initially generated ROS (Amorim et al. 2024; Vieira et al. 2025). Strong KI-mediated potentiation has been demonstrated against planktonic bacteria and biofilms (Amorim et al. 2024). This strategy is particularly relevant to difficult-to-control Gram-negative pathogens such as *Klebsiella pneumoniae*, an ESKAPE organism frequently associated with hospital-acquired infections (Wyres et al. 2020).

Beyond the treatment of localized infections, photoactive antimicrobial materials are relevant to settings in which microorganisms colonize abiotic surfaces or are dispersed in aqueous environments. Photodynamic eradication of biofilms on endotracheal tubes has been demonstrated experimentally (Amanda et al. 2018), and related approaches have been investigated in experimental models of pneumonia (Geralde et al. 2017). Visible-light-active TiO<sub>2</sub> materials have also been studied for surface and water disinfection, providing an environmental counterpart to their biomedical use (Barbosa Pires et al. 2026; Sulek et al. 2019b). Although these applications require different material formats and validation criteria, they share the need for photochemically stable systems capable of functioning under visible-light irradiation.

In the present study, colloidal nanocrystalline TiO<sub>2</sub> (qTiO<sub>2</sub>) was surface-sensitized with the free-base porphyrins TCPP and THPP, differing in their peripheral anchoring groups, the corresponding zinc(II) complex ZnTHPP, and hypericin (Hyp). The study was designed to determine how sensitizer structure influences interfacial charge transfer, ROS formation, colloidal properties, and antimicrobial performance. A central question was whether coupling molecular photosensitizers intrinsically capable of efficient <sup>1</sup>O<sub>2</sub> generation to qTiO<sub>2</sub> could combine Type II energy transfer with semiconductor-mediated electron-transfer chemistry under visible-light excitation (Setsukinai et al. 2003). The resulting materials were examined against Gram-positive *Staphylococcus aureus* and Gram-negative *Klebsiella pneumoniae*, while KI potentiation was used to test whether secondary iodine chemistry could overcome limitations imposed by the microbial envelope. By relating the physicochemical and photochemical characteristics of the sensitized materials to their antibacterial activity, the study addresses why efficient visible-light ROS generation does not necessarily translate into comparable biological efficacy.

## 2. Materials and Methods

**2.1. Synthesis and Modification of Nanomaterials** The starting material, a 5% aqueous colloidal dispersion of nanocrystalline titanium(IV) oxide (qTiO<sub>2</sub>, anatase phase) (3D-nano, Poland), was stabilized using a 1 M sodium citrate solution. To optimize colloidal stability, the qTiO<sub>2</sub> dispersion was mixed with the sodium citrate solution at volume ratios of 1:1, 1:2, and 1:3 and subsequently dialyzed for 72 h (3 × 24 h) against distilled water using 6.7 kDa cut-off dialysis tubing. Based on the initial DLS and zeta-potential measurements, the 1:2 volume ratio was selected for subsequent surface modification.

Surface sensitization was performed using TCPP, THPP, ZnTHPP (PorphyrChem SAS), and Hyp (Merck). Solutions of the porphyrin sensitizers were prepared in THF, whereas Hyp was prepared in an aqueous

Triton X-100 solution (<1%, v/v). The amount of sensitizer used for surface modification was adjusted spectrophotometrically to yield an initial absorbance of 1.0 at the respective absorption maximum of each chromophore. Briefly, the porphyrin modifiers were prepared in aqueous solutions containing < 1% (v/v) THF, whereas hypericin was prepared with < 1% (v/v) Triton X-100. These solutions were subsequently mixed with the qTiO<sub>2</sub> dispersion in a 1:1 volume ratio and subjected to dialysis (membrane cut-off 6.7 kDa) against demineralized water for 72 h (with the dialysate exchanged every 24 h).

**2.2. Nanoparticle Size and Colloidal Stability Measurements** The hydrodynamic diameter of the colloidal particles was determined by dynamic light scattering (DLS), and the zeta potential ( $\zeta$ ) was determined from electrophoretic mobility measurements. Measurements were performed directly in the colloidal dispersions at 25 °C using a Zetasizer Ultra (Malvern Instruments). Each reported value represents the mean of five consecutive measurements of the same dispersion.

**2.3. Electronic Absorption Spectroscopy** UV–Vis absorption spectra of the free photosensitizers, bare qTiO<sub>2</sub>, and functionalized qTiO<sub>2</sub> materials were recorded using a dual-beam Shimadzu UV-1900i spectrophotometer equipped with 1 cm path-length quartz cuvettes. Spectra were collected over the 300–800 nm wavelength range using the corresponding solvent or dispersion medium as the reference. The absorbance of the measured samples was maintained below 1.5.

**2.4. Determination of the *n*-Octanol/Water Partition Coefficient** The *n*-octanol/water partition coefficient (logP) was determined using mutually saturated *n*-octanol and PBS phases. A small amount of each photosensitizer was dissolved in 5 mL of PBS-saturated *n*-octanol by sonication for 5 min, after which 5 mL of *n*-octanol-saturated PBS was added. The biphasic mixture was vortexed for approximately 15 min and centrifuged at 3700 rpm for 3 min. Aliquots (20  $\mu$ L) from each phase were diluted with 3.98 mL of DMSO, sonicated for 5 min, and their fluorescence emission spectra were recorded using a PerkinElmer LS 55 spectrofluorometer. The relative fluorescence intensities of the two phases were used to determine logP.

**2.5. Estimation of Apparent Optical Gap** Apparent optical gap values ( $E_g$ ) of bare and sensitized qTiO<sub>2</sub> dispersions were estimated using the Tauc approach for an indirect transition (Tauc et al. 1966). The quantity  $(AE)^{1/2}$  was plotted as a function of photon energy ( $E$ , in eV), where  $A$  represents absorbance, and  $E_g$  was estimated by extrapolating the linear region of the plot to the energy-axis intercept. The obtained values were used as comparative optical descriptors of the dispersions.

**2.6. Dark Stability and Photostability of Photosensitizers** The dark stability of the functionalized qTiO<sub>2</sub> dispersions was evaluated after one month of storage by UV–Vis absorption spectroscopy, dynamic light scattering (DLS), and zeta-potential measurements. Changes in the absorption spectra, hydrodynamic diameter, and surface potential were used to assess their spectral and colloidal stability during storage. Photostability was evaluated by continuously irradiating the functionalized qTiO<sub>2</sub> dispersions in quartz cuvettes using a 150 W xenon lamp equipped with a 380 nm long-pass filter. UV–Vis absorption spectra were recorded over the 300–800 nm range after 0, 1, 4, 5, 10, 15, 30, and 60 min of cumulative irradiation. Photostability was quantified from the absorbance at the characteristic absorption maximum of each sensitizer and expressed as  $A_t/A_0 \times 100\%$ , where  $A_0$  and  $A_t$  represent the absorbance before irradiation and after irradiation time  $t$ , respectively.

**2.7. Steady-State Fluorescence Emission Spectroscopy** Steady-state fluorescence emission spectra of the free sensitizers and the corresponding qTiO<sub>2</sub>-based materials were recorded using a Shimadzu RF-6000 spectrofluorometer configured with a right-angle (90°) detection geometry. Emission spectra were recorded in the 550–800 nm range using a scan speed of 60 nm/min and slit widths set to 5.0 nm for both excitation and emission paths. To mitigate inner filter effects, the absorbance of all measured solutions was kept strictly at approximately 0.01 at the excitation wavelength. The excitation wavelengths were assigned as 410–430 nm for the porphyrin derivatives and approximately 515 nm for hypericin. Fluorescence quantum yields ( $\Phi_F$ ) were calculated using the comparative relative method, employing meso-tetra(4-sulfonatophenyl)porphyrin (TPPS) in water ( $\Phi_F = 0.65$ ) as the reference standard (Sulek et al. 2019a).

**2.8. Photoelectrochemical Measurements** Wavelength-dependent photocurrent responses of bare and functionalized qTiO<sub>2</sub> were measured in a three-electrode photoelectrochemical cell using a photoelectric

spectrometer (Instytut Fotonowy, Poland). Working electrodes were prepared by spreading an aqueous dispersion of the respective qTiO<sub>2</sub>-based material onto indium tin oxide (ITO)-coated transparent substrates, followed by drying in a stream of warm air. A platinum wire and Ag/AgCl electrode were used as the counter and reference electrodes, respectively, and 0.1 M aqueous KNO<sub>3</sub> served as the supporting electrolyte. The working electrodes were irradiated from the ITO side with monochromatic light in the 330–500 nm range to minimize the influence of the deposited layer thickness on the measured photocurrent. Photocurrent responses were recorded at an applied potential of +1.0 V vs Ag/AgCl.

**2.9. Determination of Singlet Oxygen Quantum Yield** Singlet oxygen generation was evaluated by photosensitized oxidation of 9,10-dimethylanthracene (DMA) or 1,3-diphenylisobenzofuran (DPBF). For the porphyrin derivatives, DMA was used in PBS, with *meso*-tetra(4-sulfonatophenyl)porphine (TPPS) serving as the reference photosensitizer ( $\Phi_{\Delta} = 0.70$ ; (Sulek et al. 2020)). The photosensitizer solution (10  $\mu$ M) was mixed with DMA and irradiated with an LED source at wavelengths corresponding to the absorption of the sensitizer. The decrease in DMA absorbance was monitored after successive irradiation intervals.

For hypericin, DPBF measurements were performed in DMF using zinc phthalocyanine (ZnPc) as the reference photosensitizer ( $\Phi_{\Delta} = 0.56$ ; (Pucelik et al. 2016b)). Hypericin and ZnPc solutions were adjusted to an absorbance of approximately 1 at their respective absorption maxima (599 nm and 673 nm, respectively). DPBF was subsequently added to yield an absorbance of approximately 1 at 417 nm. The samples were irradiated using a  $595 \pm 10$  nm LED source for hypericin, while red light with a  $> 580$  nm cut-off filter was used for the ZnPc reference. The decrease in DPBF absorbance at 417 nm was followed spectrophotometrically after successive irradiation periods. Singlet oxygen quantum yields ( $\Phi_{\Delta}$ ) were determined relative to the corresponding reference sensitizer. The values were calculated by comparing the slopes of the linear regression lines obtained from the first-order photobleaching kinetics, which were derived by plotting the natural logarithm of the sensitizer-to-probe absorbance ratio as a function of irradiation time.

**2.10. Detection of Reactive Oxygen Species (ROS)** Reactive oxygen species generated by the free photosensitizers and the corresponding qTiO<sub>2</sub>-based materials were monitored using ROS-sensitive fluorescent probes: 3-(p-aminophenyl)fluorescein (APF) for hydroxyl-radical-associated oxidation, dihydroethidium (DHE) for superoxide-associated oxidation, and Singlet Oxygen Sensor Green (SOSG) for singlet oxygen (<sup>1</sup>O<sub>2</sub>) (Gomes et al. 2005; Setsukinai et al. 2003). In a typical experiment, 100  $\mu$ L of a photosensitizer solution or functionalized qTiO<sub>2</sub> dispersion, adjusted to an initial absorbance of approximately 0.05 at the corresponding irradiation wavelength, was mixed with 1  $\mu$ L of the respective fluorescent probe stock solution (10  $\mu$ M), giving a final probe concentration of approximately 0.1  $\mu$ M. Samples were irradiated using LED sources selected according to the absorption properties of the sensitizers:  $420 \pm 20$  nm for the porphyrin-based systems and  $600 \pm 20$  nm for Hyp and Hyp@qTiO<sub>2</sub>. Fluorescence was measured using a Tecan multimode microplate reader at the following excitation and emission wavelengths  $\lambda_{\text{ex}} / \lambda_{\text{em}}$ : 488/515 nm for APF, 480/580 nm for DHE, and 505/525 nm for SOSG. Nine reading points were averaged for each well. Measurements were recorded before irradiation and after cumulative irradiation periods of 0.5, 1, 3, 5, 10, 15, and 20 min.

**2.11. In Vitro Antimicrobial Photodynamic Inactivation** The photodynamic efficacy of the synthesized nanomaterials was evaluated against Gram-positive methicillin-resistant *Staphylococcus aureus* LAC (Kwiecinski et al. 2022) and Gram-negative rifampin-resistant *Klebsiella pneumoniae* KPPR1 (Broberg et al. 2014). Bacteria from overnight cultures on tryptic soy agar (TSA) at 37 °C were suspended in phosphate-buffered saline (PBS). The functionalized TiO<sub>2</sub> suspensions were mixed in a 9:1 volume ratio (90  $\mu$ L of the material dispersion and 10  $\mu$ L of the bacterial suspension) in 96-well polystyrene plates, yielding a final inoculum of approximately  $4 \cdot 10^7$  colony-forming units per mL (CFU/mL) in a total volume of 100  $\mu$ L/well. To ensure consistency with the photochemical assays, the final absorbance of the photoactive system in the reaction mixture was adjusted to approximately 0.05. The plates were incubated in the dark for 120 min before irradiation. Following dark incubation, the samples were irradiated with a  $420 \pm 20$  nm LED light source. The light source was calibrated to deliver a light dose of 1 J/cm<sup>2</sup> per minute, and the samples were irradiated for 10 min to achieve a total light dose of 10 J/cm<sup>2</sup>. For potentiation experiments, a freshly prepared potassium iodide (KI) solution was added to selected wells immediately prior to irradiation, achieving a final KI concentration of 100 mM. A comprehensive set of controls was performed simultaneously, including bacteria alone (dark and light), material alone (dark and light), and KI alone (dark and light), to rule out inherent toxicity of the components or the light itself. Post-irradiation,

surviving bacteria were enumerated by serial dilution in PBS and plating on TSA. The results are expressed as CFU/mL, with a detection limit of  $10^2$  CFU/mL. Two independent experiments were performed, each comprising three technical replicates per condition, and the results were pooled. Data are presented as the mean  $\pm$  standard deviation (SD).

**2.12. Statistical Analysis** Statistical analysis was performed using GraphPad Prism software version 8.0.1 (GraphPad Software, San Diego, CA, USA). To satisfy the mathematical assumption of normal distribution for microbiological count data, all raw CFU/mL values were  $\log_{10}$ -transformed prior to the analysis. The statistical significance of differences between the experimental groups was evaluated using a two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. A *p*-value of less than 0.05 was considered statistically significant. Significance levels are denoted on the graphs by asterisks.

### 3. Results

#### 3.1. Molecular Structures and Spectroscopic Properties of the Photosensitizers

Four photosensitizers were selected for qTiO<sub>2</sub> surface sensitization: 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (TCPP), 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (THPP), zinc(II) 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (ZnTHPP), and hypericin (Hyp) (**Figure 1A**). TCPP and THPP differ in the peripheral groups available for interaction with the TiO<sub>2</sub> surface, whereas comparison of THPP with ZnTHPP enables assessment of the effect of Zn(II) coordination within the porphyrin macrocycle. Hypericin, a naphthodianthrone chromophore, was included as a structurally distinct, non-tetrapyrrolic photosensitizer.

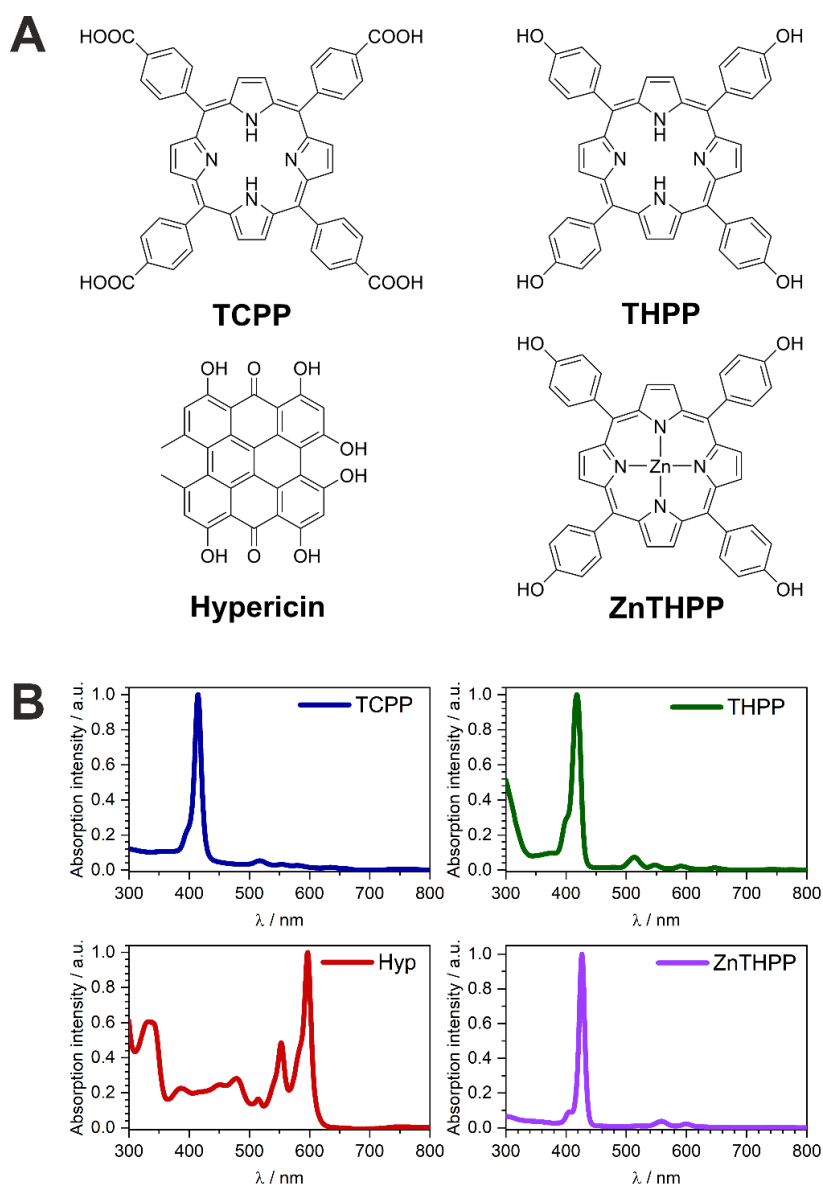


**Figure 1 (A)** Chemical structures of TCPP, THPP, ZnTHPP, and Hyp, showing the functional groups potentially involved in their interaction with the TiO<sub>2</sub> surface. **(B)** Normalized UV–Vis electronic absorption spectra of the unmodified photosensitizers.

The UV–Vis electronic absorption spectra of the free photosensitizers are shown in **Figure 1B**, and their principal spectroscopic, photophysical, and physicochemical parameters are summarized in **Table 1**. TCPP and THPP displayed the characteristic absorption pattern of free-base porphyrins, with intense Soret bands at 415 and 421 nm, respectively, followed by four lower-intensity Q bands in the visible region. Zn(II) coordination reduced the number of Q bands and shifted the Soret maximum of ZnTHPP to 426 nm, consistent with the higher symmetry of the metalloporphyrin macrocycle. Hypericin exhibited a broader vibronically structured absorption profile extending to longer wavelengths, with its longest-wavelength maximum at 595 nm.

The n-octanol/PBS partition coefficients differed markedly across the series. TCPP was the least lipophilic photosensitizer ( $\log P = 0.34$ ), whereas considerably higher values were obtained for THPP, ZnTHPP, and Hyp (2.67, 1.83, and 2.11, respectively). Fluorescence measurements of the n-octanol and PBS phases used in the partition analysis are presented in **Figure S1**. The fluorescence quantum yields, determined independently, also varied among the sensitizers, from 0.03 for ZnTHPP to 0.37 for TCPP (**Table 1**).

**Table 1** Spectroscopic, photophysical, and physicochemical properties of the free photosensitizers, including absorption and emission maxima, fluorescence quantum yields ( $\Phi_F$ ), singlet oxygen quantum yields ( $\Phi_\Delta$ ), and n-octanol/PBS partition coefficients (logP).

| PS                                           | $\lambda_{ex} / \text{nm}; \epsilon / \text{M}^{-1} \cdot \text{cm}^{-1} \cdot 10^3$ |               |                                   |               |              | $\lambda_{em} / \text{nm}$ |           | $\Phi_F$ | $\Phi_\Delta$ | logP |
|----------------------------------------------|--------------------------------------------------------------------------------------|---------------|-----------------------------------|---------------|--------------|----------------------------|-----------|----------|---------------|------|
|                                              | B                                                                                    | $Q_y$ (0-1)   | $Q_y/Q$ (0-0)                     | $Q_y/Q$ (0-1) | $Q_x$ (0-0)  | $Q$ (0-0)                  | $Q$ (0-1) |          |               |      |
| TCPP                                         | 415;<br>296,0                                                                        | 511;<br>21,47 | 541;<br>6,51                      | 587;<br>4,40  | 655;<br>3,88 | 645                        | 706       | 0.37     | 0.744         | 0.34 |
| THPP                                         | 421;<br>290,1                                                                        | 517;<br>10,43 | 554;<br>7,85                      | 596;<br>3,80  | 653;<br>3,08 | 662                        | 726       | 0.11     | 0.501         | 2.67 |
| ZnTHPP                                       | 426;<br>319,6                                                                        | -             | 558;<br>1,166                     | 599;<br>6,99  | -            | 615                        | 655       | 0.03     | 0.427         | 1.83 |
| $\pi \rightarrow \pi^*$ vibronic progression |                                                                                      |               |                                   |               |              |                            |           |          |               |      |
| Hypericin                                    |                                                                                      |               | 478,<br>514,<br>552,<br>595; 41,6 |               |              | 602                        | 649       | 0.30     | 0.43          | 2.11 |

Singlet oxygen generation by the free photosensitizers was evaluated using chemical trapping methods (**Figure S2**) (Mfouo-Tynga et al. 2021). All three porphyrins showed substantial ability to photosensitize singlet oxygen formation. The  $\Phi_\Delta$  values determined in PBS using DMA and TPPS as the reference were 0.744 for TCPP, 0.501 for THPP, and 0.427 for ZnTHPP (**Table 1; Figure S2**). Hypericin gave  $\Phi_\Delta = 0.43$  in DMF using DPBF and ZnPc as the reference photosensitizer. Because the hypericin measurement was performed using a different solvent, singlet-oxygen acceptor, and reference compound, its  $\Phi_\Delta$  value was considered separately rather than used for direct quantitative comparison with the porphyrin series.

### 3.2. Optimization and Colloidal Stability of qTiO<sub>2</sub>

Before surface modification, the colloidal stability of qTiO<sub>2</sub> was optimized by varying the volume ratio of the qTiO<sub>2</sub> dispersion to sodium citrate solution. The tested 1:1, 1:2, and 1:3 ratios yielded mean hydrodynamic diameters of 15.62, 12.21, and 19.86 nm, respectively, with corresponding zeta potentials of -37.88, -48.73, and -44.90 mV. The 1:2 formulation combined the smallest hydrodynamic diameter with the highest magnitude of negative zeta potential and was therefore selected for subsequent surface sensitization.

The selected qTiO<sub>2</sub> dispersion was additionally characterized after one month of storage in the dark. Its mean hydrodynamic diameter remained essentially unchanged (12.21 nm initially and 11.65 nm after one month), whereas the zeta potential changed from -48.73 to -36.19 mV (**Table 2**). Consistent with the mean diameter values, the DLS size distributions recorded immediately after preparation and after storage showed no marked shift toward larger particle populations (**Figure S3**).

**Table 2** Hydrodynamic diameter (D) and zeta potential ( $\zeta$ ) of bare and functionalized qTiO<sub>2</sub> dispersions immediately after preparation and after one month of storage in the dark.

| Material                 | $\zeta / \text{mV}$ (Initial) | $\zeta / \text{mV}$ (1 month) | D / nm (Initial) | D / nm (1 month) |
|--------------------------|-------------------------------|-------------------------------|------------------|------------------|
| qTiO <sub>2</sub>        | -48.73                        | -36.19                        | 12.21            | 11.65            |
| TCPP@qTiO <sub>2</sub>   | -46.48                        | -45.03                        | 20.57            | 15.72            |
| THPP@qTiO <sub>2</sub>   | -31.46                        | -38.69                        | 20.03            | 17.97            |
| ZnTHPP@qTiO <sub>2</sub> | -29.10                        | -38.63                        | 32.83            | 27.48            |
| Hyp@qTiO <sub>2</sub>    | -25.12                        | -34.30                        | 20.81            | 37.88            |

### 3.3. Surface Sensitization and Colloidal Properties of Functionalized qTiO<sub>2</sub>

The citrate-stabilized qTiO<sub>2</sub> dispersion was sensitized with TCPP, THPP, ZnTHPP, or Hyp. Following mixing and subsequent dialysis, absorption bands characteristic of the respective chromophores remained detectable in all qTiO<sub>2</sub>-based dispersions (**Figure S4**), indicating retention of the sensitizers within the colloidal systems after

removal of low-molecular-weight components. Surface sensitization introduced distinct absorption features in the visible region (**Table 3**). TCPP@qTiO<sub>2</sub> showed maxima at 406, 518, 595, and 650 nm; THPP@qTiO<sub>2</sub> at 423, 523, 575, and 655 nm; ZnTHPP@qTiO<sub>2</sub> at 434, 564, and 605 nm; and Hyp@qTiO<sub>2</sub> at 477, 516, 553, and 597 nm. Thus, all functionalized dispersions retained characteristic sensitizer absorption extending well beyond the intrinsic absorption range of bare qTiO<sub>2</sub>.

Tauc analysis yielded apparent optical gap values of 3.37, 3.39, 3.35, and 3.40 eV for TCPP@qTiO<sub>2</sub>, THPP@qTiO<sub>2</sub>, ZnTHPP@qTiO<sub>2</sub>, and Hyp@qTiO<sub>2</sub>, respectively, compared with 3.50 eV for bare qTiO<sub>2</sub> (**Figure S5**). Because these values were derived from absorption spectra of colloidal sensitized systems, they are used here as comparative optical descriptors rather than as evidence for modification of the intrinsic band structure of anatase TiO<sub>2</sub> (Tauc et al. 1966).

**Table 3** Visible absorption maxima ( $\lambda_{\text{max}}$ ) and apparent optical gap values ( $E_g$ ) of bare and sensitized qTiO<sub>2</sub> dispersions.

| Material          | $\lambda_{\text{max}}$ / nm | $E_g$ [eV] |
|-------------------|-----------------------------|------------|
| qTiO <sub>2</sub> | -                           | 3.50       |
| TCPP              | 406, 518, 595, 650          | 3.37       |
| THPP              | 423, 523, 575, 655          | 3.39       |
| ZnTHPP            | 434, 564, 605               | 3.35       |
| Hyp               | 477, 516, 553, 597          | 3.40       |

The freshly prepared functionalized dispersions showed mean hydrodynamic diameters between approximately 20 and 33 nm (**Table 2**). TCPP@qTiO<sub>2</sub> and THPP@qTiO<sub>2</sub> exhibited similar mean diameters of 20.57 and 20.03 nm, respectively, whereas ZnTHPP@qTiO<sub>2</sub> showed a somewhat larger value of 32.83 nm. The initial mean diameter of Hyp@qTiO<sub>2</sub> was 20.81 nm. All functionalized materials exhibited negative zeta potentials, although their magnitude depended on the sensitizer. TCPP@qTiO<sub>2</sub> retained a zeta potential close to that of bare qTiO<sub>2</sub> (−46.48 mV), whereas less negative values were measured for THPP@qTiO<sub>2</sub> (−31.46 mV), ZnTHPP@qTiO<sub>2</sub> (−29.10 mV), and Hyp@qTiO<sub>2</sub> (−25.12 mV).

After one month of storage in the dark, no increase in mean hydrodynamic diameter was observed for the three porphyrin-functionalized dispersions, with values of 15.72, 17.97, and 27.48 nm for TCPP@qTiO<sub>2</sub>, THPP@qTiO<sub>2</sub>, and ZnTHPP@qTiO<sub>2</sub>, respectively. In contrast, the mean diameter of Hyp@qTiO<sub>2</sub> increased from 20.81 to 37.88 nm. Zeta potentials remained negative for all systems after storage (**Table 2**). Complete DLS distributions and UV–Vis spectra recorded before and after storage are provided in **Figure S6**.

### 3.4. Steady-State Fluorescence Properties

Under matched absorbance conditions at the excitation wavelength, surface sensitization of qTiO<sub>2</sub> produced compound-dependent changes in fluorescence emission (**Figure 2**). Fluorescence emission was markedly quenched for TCPP, THPP, and ZnTHPP after association with qTiO<sub>2</sub>, whereas Hyp fluorescence was affected to a much smaller extent. Changes in the positions of the emission maxima were also sensitizer-dependent (**Table 4**). TCPP showed the most pronounced spectral shift, with its emission maxima changing from 645 and 706 nm for the free sensitizer to 656 and 725 nm for TCPP@qTiO<sub>2</sub>. THPP showed only minor changes, from 662 and 726 nm to 661 and 727 nm, respectively. For ZnTHPP, the maxima shifted from 615 and 655 nm to 617 and 667 nm.

The emission maxima of Hyp remained essentially unchanged, at 602 and 649 nm for the free sensitizer and 601 and 649 nm after association with qTiO<sub>2</sub>.

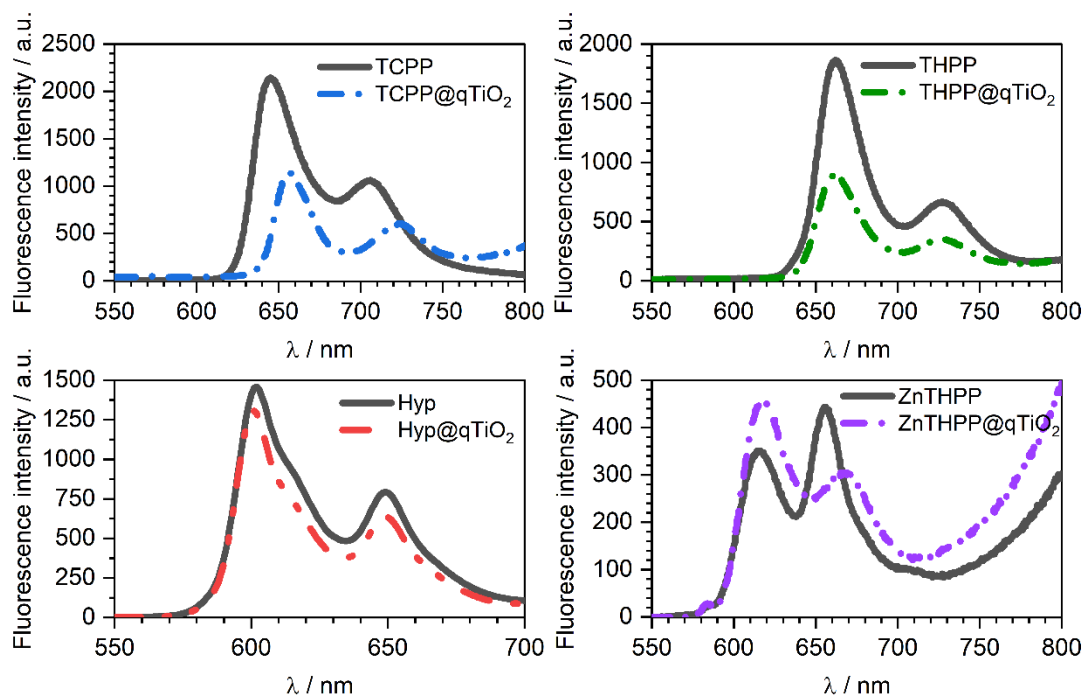


**Figure 2** Steady-state fluorescence emission spectra of the free photosensitizers and the corresponding functionalized qTiO<sub>2</sub> materials.

**Table 4** Emission maxima of the free photosensitizers and functionalized qTiO<sub>2</sub> materials and fluorescence quantum yields ( $\Phi_F$ ) of the free photosensitizers.

| Photosensitizer (PS) | Free PS $\lambda_{em}$ / nm | Functionalized qTiO <sub>2</sub><br>$\lambda_{em}$ / nm | $\Phi_F$ |
|----------------------|-----------------------------|---------------------------------------------------------|----------|
| TCPP                 | 645, 706                    | 656, 725                                                | 0.37     |
| THPP                 | 662, 726                    | 661, 727                                                | 0.11     |
| ZnTHPP               | 615, 655                    | 617, 667                                                | 0.03     |
| Hyp                  | 602, 649                    | 601, 649                                                | 0.30     |

### 3.5. Photostability of Functionalized qTiO<sub>2</sub> Materials

Changes in the characteristic absorption bands of the functionalized qTiO<sub>2</sub> materials during 60 min of visible-light irradiation are shown in **Figure 3**. All investigated materials retained more than 80% of their initial absorbance after 60 min. Hyp@qTiO<sub>2</sub> showed the highest absorbance retention (94.40%), followed by TCPP@qTiO<sub>2</sub> (91.72%), ZnTHPP@qTiO<sub>2</sub> (85.79%), and THPP@qTiO<sub>2</sub> (82.49%) (**Table 5**).

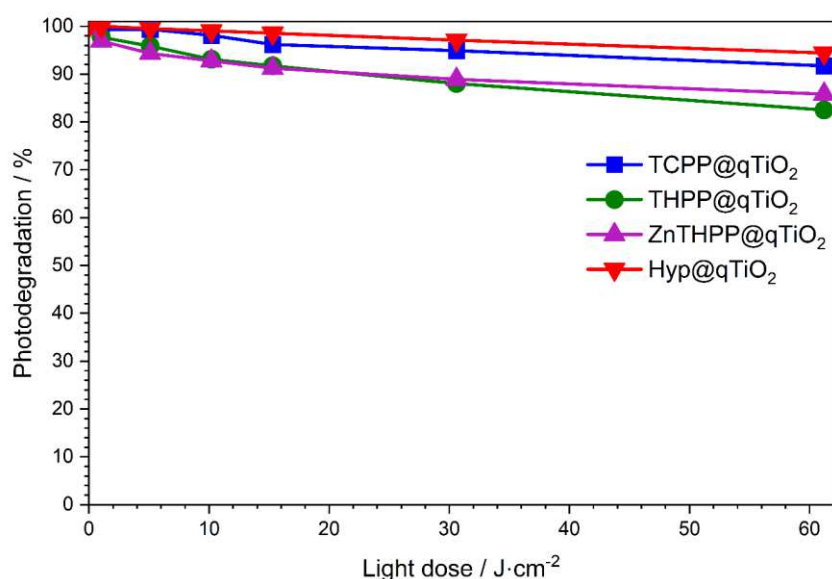


**Figure 3** Photostability of the functionalized qTiO<sub>2</sub> materials expressed as relative absorbance ( $A_t/A_0 \times 100\%$ ) during visible-light irradiation.

The complete UV–Vis absorption spectra recorded during irradiation are provided in **Figure S7**. In all cases, the characteristic sensitizer-associated absorption bands remained detectable throughout the experiment, with no abrupt loss of the principal spectral features.

**Table 5** Absorbance retention of the functionalized qTiO<sub>2</sub> materials after 60 min of visible-light irradiation.

| Material                 | $\lambda_{\max}$ / nm | $A_{\max 0}$ | $A_{\max 60}$ | $A_{60}/A_0 \times 100$ / % |
|--------------------------|-----------------------|--------------|---------------|-----------------------------|
| TCPP@qTiO <sub>2</sub>   | 406                   | 0.157        | 0.144         | 91.72                       |
| THPP@qTiO <sub>2</sub>   | 423                   | 0.217        | 0.179         | 82.49                       |
| ZnTHPP@qTiO <sub>2</sub> | 434                   | 0.387        | 0.332         | 85.79                       |
| Hyp@qTiO <sub>2</sub>    | 597                   | 0.410        | 0.388         | 94.40                       |

### 3.6. Photoelectrochemical Response

Wavelength-dependent photocurrent responses of bare and functionalized qTiO<sub>2</sub> are shown in **Figure 4**. Bare qTiO<sub>2</sub> exhibited its principal photoresponse in the near-UV region, consistent with excitation of the semiconductor. Surface sensitization modified the wavelength-dependent photocurrent response, with additional contributions observed toward the visible region. These changes were most pronounced for TCPP@qTiO<sub>2</sub> and THPP@qTiO<sub>2</sub>, whereas ZnTHPP@qTiO<sub>2</sub> and Hyp@qTiO<sub>2</sub> showed weaker visible-light responses under the applied experimental conditions.

The altered photoelectrochemical response overlapped with the absorption range of the surface-associated sensitizers, indicating a contribution of chromophore excitation to charge generation in the functionalized systems. Because the absolute photocurrent values may also depend on the amount and morphology

of material deposited on the ITO electrode, the measurements were used primarily to compare the wavelength dependence of the photoresponse rather than to derive quantitative electron-transfer efficiencies.

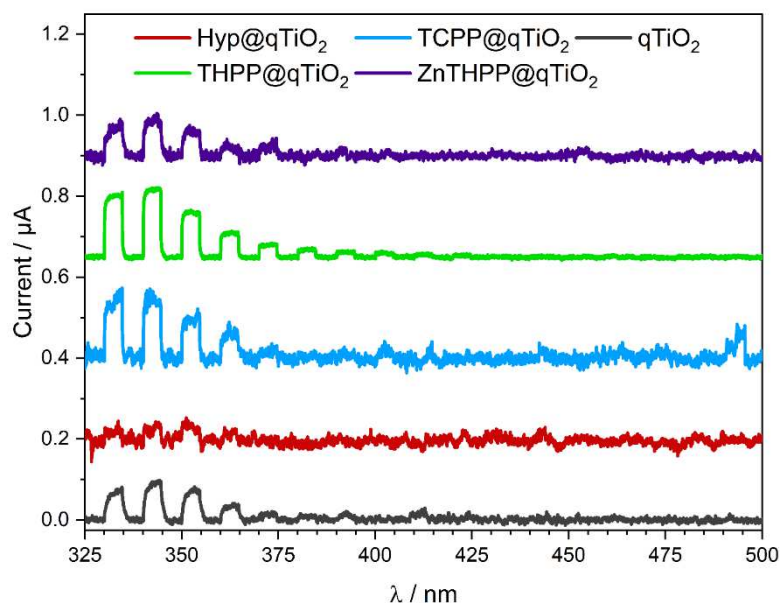


**Figure 4** Wavelength-dependent photocurrent responses of bare and functionalized qTiO<sub>2</sub> recorded at an applied potential of +1.0 V vs Ag/AgCl.

### 3.7. Reactive Oxygen Species Generation

Visible-light generation of ROS by bare qTiO<sub>2</sub>, the free photosensitizers, and the corresponding functionalized materials was monitored using APF, DHE, and SOSG (**Figure 5** and **Figure S8**). All surface-sensitized qTiO<sub>2</sub> systems produced stronger APF and DHE fluorescence than bare qTiO<sub>2</sub>, indicating increased formation of oxygen-centred radical products under irradiation. SOSG fluorescence increased with irradiation time for all functionalized materials, demonstrating that singlet oxygen generation was retained after association of the sensitizers with qTiO<sub>2</sub>.

Comparison with the corresponding free photosensitizers revealed different changes in ROS generation after surface sensitization. TCPP@qTiO<sub>2</sub>, ZnTHPP@qTiO<sub>2</sub>, and Hyp@qTiO<sub>2</sub> produced stronger APF and DHE fluorescence but lower SOSG signals than their respective free sensitizers. THPP@qTiO<sub>2</sub> displayed a different profile, with increased fluorescence of all three probes. The functionalized systems therefore generated both oxygen-centred radicals and singlet oxygen under visible-light irradiation, although the relative contribution of these ROS depended on the sensitizer. Direct comparisons between the free photosensitizers and the corresponding qTiO<sub>2</sub>-based materials are provided in **Figure S8**.

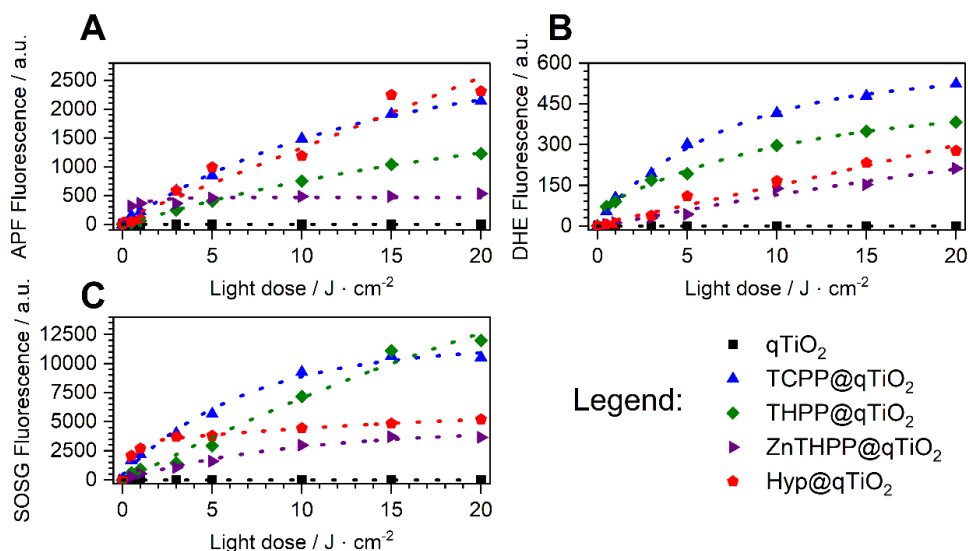


**Figure 5** Time-dependent ROS-sensitive fluorescence responses of bare and functionalized qTiO<sub>2</sub> materials under visible-light irradiation. APF was used to monitor hydroxyl-radical-associated oxidation, DHE to monitor superoxide-associated oxidation, and SOSG to monitor singlet oxygen. Porphyrin-based systems were irradiated at  $420 \pm 20$  nm and Hyp-based systems at  $600 \pm 20$  nm.

### 3.8. Photodynamic Inactivation of Bacteria

Before microbiological evaluation, the colloidal behavior of the functionalized qTiO<sub>2</sub> materials was examined under the bacterial assay conditions. The porphyrin-functionalized dispersions, TCPP@qTiO<sub>2</sub>, THPP@qTiO<sub>2</sub>, and ZnTHPP@qTiO<sub>2</sub>, showed no visible signs of destabilization under the assay conditions, whereas Hyp@qTiO<sub>2</sub> underwent aggregation after transfer to the bacterial medium. Hyp@qTiO<sub>2</sub> was nevertheless retained in the antimicrobial experiments to examine how this change in dispersion state affected its activity.

The antimicrobial activity of bare and functionalized qTiO<sub>2</sub> materials was evaluated against Gram-positive *Staphylococcus aureus* and Gram-negative *Klebsiella pneumoniae* using  $420 \pm 20$  nm irradiation at a light dose of  $10 \text{ J cm}^{-2}$  (**Figure 6**). No appreciable antibacterial activity was observed for the functionalized materials in the dark. Bare qTiO<sub>2</sub> also showed no measurable photodynamic activity under the applied visible-light conditions.

The functionalized materials differed markedly in their activity against *S. aureus*. THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub> showed strong photodynamic activity, reducing viable counts by approximately 5 and >4 log units, respectively, relative to irradiated bare qTiO<sub>2</sub>. TCPP@qTiO<sub>2</sub> produced less than a 1-log reduction, whereas Hyp@qTiO<sub>2</sub> showed only limited activity under the same  $420 \pm 20$  nm irradiation conditions.

The response of *K. pneumoniae* was distinctly different. In the absence of KI, none of the functionalized qTiO<sub>2</sub> materials produced a substantial photodynamic effect against *K. pneumoniae* at  $10 \text{ J cm}^{-2}$  (**Figure 6**).

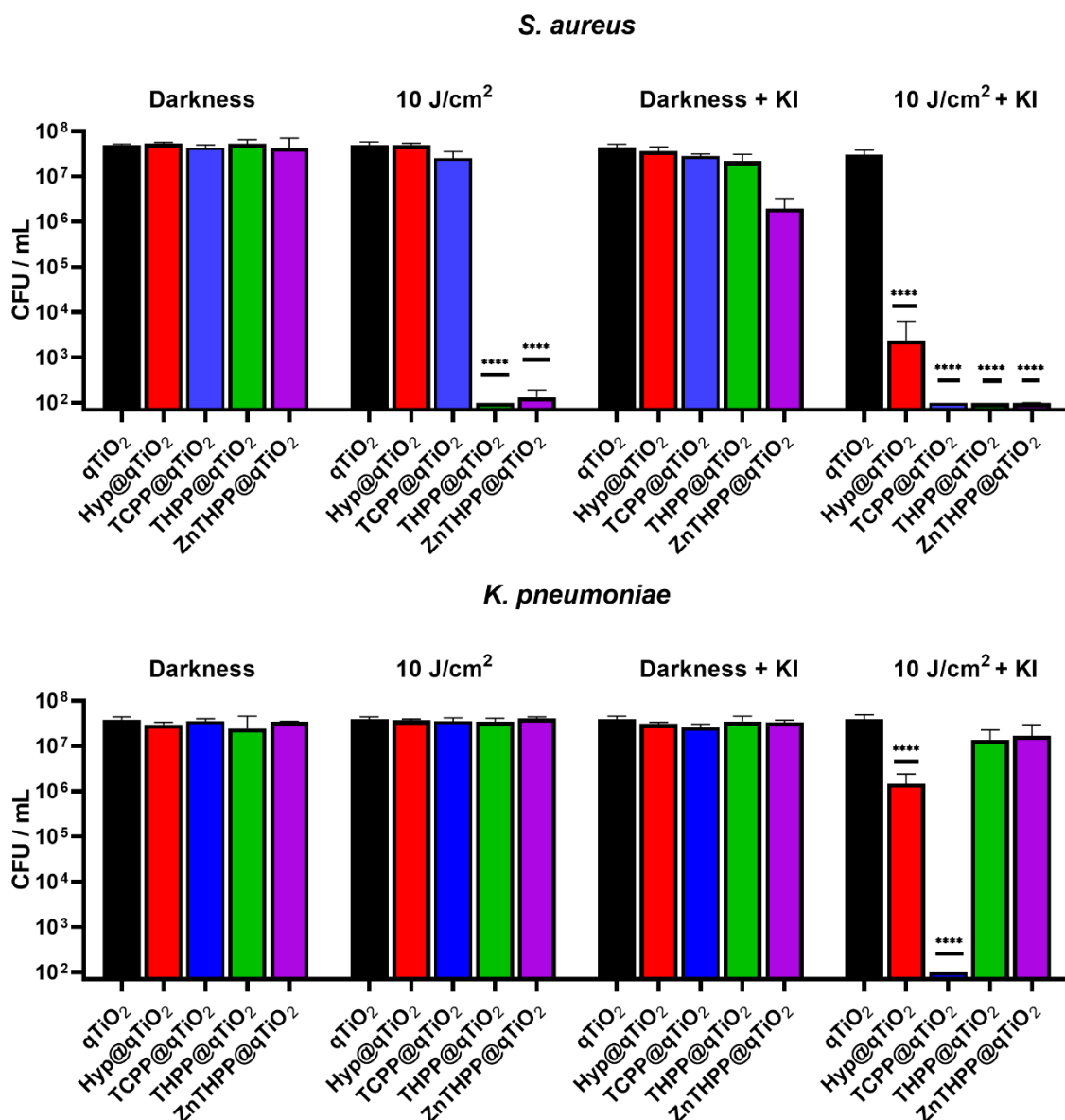


**Figure 6** Photodynamic inactivation of *S. aureus* and *K. pneumoniae* by bare and functionalized qTiO<sub>2</sub> materials in the absence and presence of potassium iodide (KI). Bacterial survival is expressed as CFU mL<sup>-1</sup> after dark incubation or irradiation with 420 ± 20 nm light at a dose of 10 J cm<sup>-2</sup>. KI was used at a final concentration of 100 mM. The detection limit was 10<sup>2</sup> CFU mL<sup>-1</sup>. Data are presented as mean ± SD from two independent experiments, each performed with three technical replicates. \*Asterisks indicate statistically significant differences relative to the corresponding control under the same irradiation and KI conditions: \*\*\*\*p < 0.0001.

Addition of 100 mM KI immediately before irradiation substantially modified the antibacterial response, but the extent of potentiation depended strongly on the sensitizer. For *S. aureus*, KI enhanced the photodynamic activity of all sensitized qTiO<sub>2</sub> materials. Following irradiation, viable counts for TCPP@qTiO<sub>2</sub>, THPP@qTiO<sub>2</sub>, and ZnTHPP@qTiO<sub>2</sub> reached the detection limit. Hyp@qTiO<sub>2</sub> also showed a pronounced KI-dependent increase in activity, although residual viable bacteria remained detectable.

The effect of KI was even more discriminating for *K. pneumoniae*. TCPP@qTiO<sub>2</sub>, which was essentially inactive against this strain in the absence of KI, showed the strongest potentiated response and reduced viable counts to the detection limit. Hyp@qTiO<sub>2</sub> produced an approximately 1.5-log reduction in viable counts, whereas THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub> remained largely ineffective despite KI addition. The magnitude of KI

potentiation therefore depended on both the sensitizer and the bacterial strain, with the strongest effect against *K. pneumoniae* observed for TCPP@qTiO<sub>2</sub>.

#### 4. Discussion

Surface sensitization of colloidal TiO<sub>2</sub> with TCPP, THPP, ZnTHPP, and Hyp yielded dispersions that retained the characteristic visible-light absorption of the respective chromophores while remaining within the nanometric size range in water. The changes in hydrodynamic diameter and zeta potential after sensitization show that association of the chromophore with qTiO<sub>2</sub> altered not only the optical properties but also the physicochemical characteristics of the organic–inorganic interface. This is particularly relevant for the three porphyrin-based systems, which share a closely related macrocyclic scaffold but differ in the nature of the peripheral anchoring groups and, for ZnTHPP, in metal coordination. In sensitized TiO<sub>2</sub> systems, the mode of surface association, chromophore orientation, electronic coupling, and distance from the semiconductor can strongly influence charge-transfer and excited-state deactivation pathways (Monteiro et al. 2019; Nikoloudakis et al. 2022). The relatively small differences in the apparent optical gap values derived from the Tauc analysis are therefore considered here as comparative characteristics of the sensitized dispersions rather than evidence for modification of the intrinsic electronic band structure of anatase TiO<sub>2</sub>.

The fluorescence and photoelectrochemical data provide complementary evidence for sensitizer-dependent electronic coupling with qTiO<sub>2</sub>. Association with qTiO<sub>2</sub> markedly decreased the fluorescence intensity of TCPP, THPP, and ZnTHPP, indicating the introduction of additional non-radiative deactivation pathways, whereas Hyp showed substantially smaller changes. In porphyrin-sensitized TiO<sub>2</sub> systems, fluorescence quenching is commonly associated with interfacial charge-transfer processes, including electron injection from the photoexcited chromophore into acceptor states of TiO<sub>2</sub> (Malec et al. 2023; Sulek et al. 2019a; Sulek et al. 2019b). The wavelength-dependent photocurrents are consistent with such electronic communication between the chromophore and semiconductor, with the clearest changes observed for TCPP@qTiO<sub>2</sub> and THPP@qTiO<sub>2</sub> (Monteiro et al. 2019; Serpa et al. 2008). Although the absolute photocurrent amplitudes cannot be used to derive quantitative electron-injection efficiencies because they also depend on electrode loading and film morphology, the combined fluorescence and photoelectrochemical results, particularly when considered together with the ROS measurements discussed below, strongly support photoinduced interfacial electron transfer in the sensitized qTiO<sub>2</sub> systems.

The functionalized qTiO<sub>2</sub> systems retained most of their characteristic chromophore absorption during irradiation, with 82.49–94.40% of the initial absorbance remaining after 60 min. Hyp@qTiO<sub>2</sub> and TCPP@qTiO<sub>2</sub> showed the highest absorbance retention, whereas THPP@qTiO<sub>2</sub> underwent the largest, although still limited, decrease. Photobleaching can progressively reduce the effective concentration of a photoactive chromophore and thereby limit photodynamic performance (Allison and Moghissi 2013; Spikes 1992). This issue has also been observed in previously studied dye-sensitized TiO<sub>2</sub> systems, in which high initial photoactivity was not necessarily accompanied by long-term photostability (Sulek et al. 2019b; Trochowski et al. 2023). Under the conditions used here, however, no rapid loss of the principal absorption bands occurred. Differences in photostability are therefore unlikely to explain the markedly different ROS profiles or antibacterial activities of the porphyrin-functionalized materials.

The ROS measurements provided further evidence that molecular and semiconductor-mediated photochemistry coexist in the sensitized materials. The free porphyrins were substantial singlet-oxygen sensitizers, with  $\Phi_{\Delta}$  values of 0.744, 0.501, and 0.427 for TCPP, THPP, and ZnTHPP, respectively. At the same time, all four surface-sensitized qTiO<sub>2</sub> systems produced stronger APF and DHE signals than bare qTiO<sub>2</sub>, consistent with enhanced formation of oxygen-centred radical products under visible-light irradiation. Compared with the corresponding free photosensitizers, TCPP@qTiO<sub>2</sub>, ZnTHPP@qTiO<sub>2</sub>, and Hyp@qTiO<sub>2</sub> produced stronger APF and DHE signals but lower SOSG fluorescence. Together with fluorescence quenching and the wavelength-dependent photocurrent measurements, these changes strongly support the opening of an interfacial electron-transfer pathway upon association with qTiO<sub>2</sub>. This pathway operates alongside the intrinsic molecular photochemistry of the sensitizers, which in the porphyrin series includes efficient energy transfer to O<sub>2</sub> and formation of <sup>1</sup>O<sub>2</sub>. In porphyrin-sensitized TiO<sub>2</sub> systems, this pathway is consistent with electron injection from the photoexcited chromophore into acceptor states of the semiconductor, followed by reduction of molecular oxygen and formation of oxygen-centred radical species (Baptista et al. 2017; Sulek et al. 2019a; Sulek et al.

2019b). The ROS-probe data alone do not permit quantitative partitioning between Type I and Type II processes, but their agreement with the fluorescence and photoelectrochemical measurements supports an additional electron-transfer route leading to oxygen-centred radicals while singlet-oxygen sensitization remains operative.

THPP@qTiO<sub>2</sub> displayed a distinct photochemical profile. In this system, stronger APF and DHE signals were accompanied by increased SOSG fluorescence relative to free THPP. Thus, access to interfacial electron-transfer chemistry did not suppress singlet-oxygen generation by THPP@qTiO<sub>2</sub>. THPP@qTiO<sub>2</sub> therefore retained substantial Type II photochemistry while simultaneously gaining access to radical-forming pathways through the qTiO<sub>2</sub> interface. The coexistence of these pathways is relevant to antimicrobial PDI because the biological effect depends not only on the amount of ROS generated but also on their identity, lifetime, diffusion range, and spatial relationship to susceptible cellular targets. Previous studies have similarly shown that antimicrobial activity of porphyrinoid photosensitizers cannot be predicted from a single photochemical parameter, but instead reflects the combined effects of ROS generation, charge, lipophilicity, aggregation, and association with microbial cells (Barczyk et al. 2026; Malec et al. 2023; Pucelik and Dąbrowski 2022; Serpa et al. 2008; Sułek et al. 2020).

Comparison with the antibacterial data shows that the photochemical differences alone do not determine biological activity. Under irradiation without KI, THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub> showed strong activity against *S. aureus*, reducing viable counts by approximately 5 and >4 log units, respectively, relative to irradiated bare qTiO<sub>2</sub>. TCPP@qTiO<sub>2</sub>, in contrast, was essentially ineffective despite producing strong APF and DHE signals. The biological ranking therefore clearly diverged from the ROS-generating capacity measured in the cell-free system. This divergence can be rationalized by considering the physicochemical properties that distinguish the three porphyrin-based materials. TCPP@qTiO<sub>2</sub> and THPP@qTiO<sub>2</sub> had nearly identical initial hydrodynamic diameters (20.57 and 20.03 nm, respectively), arguing against particle size as the principal explanation for their markedly different activity. Their interfacial properties, however, differed substantially. TCPP@qTiO<sub>2</sub> retained a strongly negative zeta potential of −46.48 mV, compared with −31.46 mV for THPP@qTiO<sub>2</sub> and −29.10 mV for ZnTHPP@qTiO<sub>2</sub>. Moreover, the corresponding free sensitizers differed markedly in lipophilicity, with TCPP being considerably more hydrophilic (logP = 0.34) than THPP (2.67) or ZnTHPP (1.83). Charge, lipophilicity, and supramolecular organization are established determinants of photosensitizer association with bacterial envelopes and can strongly modulate antimicrobial efficacy independently of intrinsic ROS production (Pucelik and Dąbrowski 2022; Sułek et al. 2020). The strongly negative surface potential of TCPP@qTiO<sub>2</sub>, together with the low lipophilicity of the parent TCPP, may therefore disfavor sufficiently close association with the negatively charged *S. aureus* envelope. In contrast, the less negative surface potentials of THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub>, combined with the greater lipophilicity of their parent sensitizers, may favor localization of photochemical damage closer to bacterial targets. Because nanomaterial binding to bacterial cells was not measured directly, this interpretation should be regarded as a physicochemically supported explanation rather than a demonstrated binding mechanism.

The lack of substantial activity against *K. pneumoniae* in the absence of KI provides an additional illustration of this spatial constraint. The Gram-negative envelope contains an outer membrane rich in lipopolysaccharide that imposes a substantially stronger permeability and association barrier than the porous peptidoglycan-rich envelope of Gram-positive bacteria. For a nanomaterial that generates short-lived ROS at or near its surface, efficient photochemistry in the surrounding solution is therefore insufficient unless reactive intermediates are produced sufficiently close to susceptible bacterial structures. This provides a plausible explanation for why even THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub>, which were highly active against *S. aureus*, were ineffective against *K. pneumoniae* under the same irradiation conditions (Cieplik et al. 2018; Hamblin and Hasan 2004; Pucelik and Dąbrowski 2022).

To overcome this limitation, potassium iodide (KI) was introduced as an inorganic potentiator. Iodide can react with primary photogenerated ROS to form secondary reactive iodine species (RIS), converting short-lived oxygen-derived reactivity into chemically distinct antimicrobial oxidants. In the present systems, both oxygen-centred radicals and <sup>1</sup>O<sub>2</sub> may contribute to iodide oxidation. Hydroxyl radicals can oxidize I<sup>−</sup> to I<sup>•</sup>, while <sup>1</sup>O<sub>2</sub> provides an additional route to iodine-radical formation. The initially formed iodine radicals rapidly react with excess iodide to generate I<sub>2</sub><sup>•−</sup>, which can subsequently give molecular iodine and other longer-lived iodine-containing oxidants (Pucelik and Dąbrowski 2022):





In the presence of excess iodide, molecular iodine can additionally equilibrate with  $I^-$  to form  $I_3^-$ . Such longer-lived iodine-derived species can extend antimicrobial oxidation beyond the immediate spatial range of the primary ROS. Importantly, reactive iodine species were not measured directly in the present study; their involvement is therefore inferred from the characteristic KI-dependent potentiation and from the established chemistry of iodide-assisted PDI.

The addition of KI markedly altered the relationship between material properties and antibacterial efficacy. Against *S. aureus*, KI further increased the already strong activity of THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub>, reducing recoverable bacterial counts to the detection limit. More strikingly, TCPP@qTiO<sub>2</sub>, which was essentially ineffective without KI, also reduced *S. aureus* viability to the detection limit after potentiation, corresponding to a reduction of at least 5 log units relative to the irradiated qTiO<sub>2</sub> + KI control. The change in activity was even more discriminating for *K. pneumoniae*. TCPP@qTiO<sub>2</sub> + KI reduced the viable population to the detection limit (>5.5-log<sub>10</sub> reduction relative to the corresponding qTiO<sub>2</sub> + KI control), whereas THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub> remained largely ineffective. Thus, KI did not simply amplify the activity of all sensitized materials but changed their relative biological ranking.

The behavior of TCPP@qTiO<sub>2</sub> helps explain this change in biological activity. This material combines the most negative surface potential among the porphyrin-functionalized systems (−46.48 mV) and the low lipophilicity of the parent TCPP (logP = 0.34) with pronounced APF- and DHE-associated photochemistry, a clear photoelectrochemical response, and high photostability. Its physicochemical characteristics may therefore limit sufficiently close association with the negatively charged bacterial envelope and spatially separate the site of primary ROS generation from susceptible cellular targets. This limitation appears particularly important for direct PDI, as illustrated by the negligible activity of TCPP@qTiO<sub>2</sub> against *S. aureus* without KI. Because nanomaterial–cell association was not measured directly, however, this interpretation should be regarded as a mechanistically supported explanation rather than a demonstrated binding mechanism.

In the presence of iodide, these photochemical properties lead to a different biological outcome. The strong radical-generating activity of TCPP@qTiO<sub>2</sub>, together with evidence for interfacial electron transfer, provides primary oxidants capable of initiating secondary iodine chemistry in the surrounding medium. Conversion of short-lived ROS into iodine-derived oxidants reduces the requirement for immediate nanomaterial–cell proximity and provides a plausible explanation for the particularly strong KI-dependent activity of TCPP@qTiO<sub>2</sub> against *K. pneumoniae*. This behavior is consistent with previous studies showing that KI can markedly potentiate otherwise poorly active anionic porphyrins and TiO<sub>2</sub>-based photocatalytic systems, including against Gram-negative bacteria (Huang et al. 2018; Sufek et al. 2019a; Sufek et al. 2019b).

The contrasting behavior of THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub> further supports this interpretation. Both materials were highly effective in direct PDI of *S. aureus* and differed from TCPP@qTiO<sub>2</sub> in having less negative surface potentials; their parent photosensitizers were also substantially more lipophilic than TCPP. Yet these advantages did not translate into effective killing of *K. pneumoniae*, even after KI addition. The strong activity of THPP@qTiO<sub>2</sub> against *S. aureus* coincided with simultaneous generation of oxygen-centred radicals and <sup>1</sup>O<sub>2</sub>. The comparison therefore points to two modes of antimicrobial action: direct, cell-proximal PDI by THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub> against the more accessible Gram-positive bacterium, and KI-dependent chemical relay of photogenerated reactivity, which becomes particularly effective for TCPP@qTiO<sub>2</sub> when the Gram-negative outer membrane imposes a stronger barrier.

This distinction may also be relevant to applications in which intimate contact between a photoactive material and every microbial cell cannot be ensured. Iodide potentiation has been investigated in TiO<sub>2</sub>-based antimicrobial photocatalysis as well as in environmental and aquaculture-oriented PDI, where conversion of short-lived primary ROS into secondary antimicrobial species may extend the effective range of photochemical damage (Monteiro et al. 2019; Vieira et al. 2025). These results indicate that the chemical environment surrounding the photoactive material, together with its intrinsic photochemical properties, can strongly influence antimicrobial performance.

Finally, Hyp@qTiO<sub>2</sub> illustrates a different limitation of the nanomaterial platform. The material retained visible-light absorption, showed high photostability, and generated both radical ROS and singlet oxygen after association with qTiO<sub>2</sub>. Its biological performance, however, cannot be interpreted on the same basis as that of the three porphyrin-functionalized materials. Hyp@qTiO<sub>2</sub> underwent pronounced aggregation under the bacterial assay conditions, demonstrating that colloidal stability established in water does not necessarily persist after transfer to the microbiological assay medium. In addition, the antimicrobial experiments were performed using 420 ± 20 nm irradiation to maintain identical conditions across the material series, whereas the principal absorption of Hyp occurs at substantially longer wavelengths and its ROS-generating properties were evaluated at 600 ± 20 nm. Its limited direct photodynamic activity therefore likely reflects a combination of altered colloidal organization and suboptimal optical excitation rather than intrinsically weak photochemistry (Agostinis et al. 2002; Mfouo-Tynga et al. 2021). KI potentiation nevertheless produced an almost 5-log reduction in *S. aureus* viability and an approximately 1.5-log reduction in *K. pneumoniae*, indicating that secondary iodine chemistry can extend the biological consequences of the remaining photoactivity even when the primary photodynamic process is compromised.

The present study shows a clear distinction between photochemical activity and antibacterial efficacy in sensitized TiO<sub>2</sub> nanomaterials. Previous studies established that porphyrin sensitization can extend the photoresponse of TiO<sub>2</sub> into the visible region and promote interfacial electron transfer, ROS generation, and antimicrobial photocatalysis (Malec et al. 2023; Sulek et al. 2019a; Sulek et al. 2019b). The current results show that these properties alone are insufficient to determine antibacterial performance. THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub> were highly effective in direct PDI of the more accessible Gram-positive *S. aureus* envelope, whereas TCPP@qTiO<sub>2</sub> remained essentially inactive despite pronounced radical-associated photochemistry. Addition of iodide fundamentally changed this relationship, converting TCPP@qTiO<sub>2</sub> into the most effective system against the otherwise resistant Gram-negative *K. pneumoniae*. The biological outcome is therefore governed by the interplay between sensitizer–semiconductor photochemistry, surface and colloidal properties, spatial localization of primary ROS generation relative to microbial targets, and the surrounding chemical environment. Effective antimicrobial performance requires optimization not only of ROS-generating photochemistry but also of the interface that determines where and in what chemical form this reactivity reaches the microbial target.

## 5. Conclusions

Surface sensitization of colloidal nanocrystalline TiO<sub>2</sub> with TCPP, THPP, ZnTHPP, and hypericin produced nanoscale dispersions with extended visible-light absorption and photochemical properties distinct from those of bare qTiO<sub>2</sub>. The free porphyrins were efficient singlet-oxygen sensitizers, whereas their association with qTiO<sub>2</sub> introduced additional pathways leading to oxygen-centred radical formation. Fluorescence quenching, wavelength-dependent photocurrent generation, and ROS-probe measurements collectively support the involvement of interfacial electron transfer in this process. Importantly, singlet-oxygen generation was preserved in the functionalized materials, with THPP@qTiO<sub>2</sub> showing particularly strong contributions from both Type I and Type II photochemistry. Photosensitization of qTiO<sub>2</sub> therefore provides access to molecular energy-transfer and semiconductor-mediated redox pathways within the same visible-light-responsive system.

The antibacterial experiments showed that these photochemical properties alone did not determine biological activity. Without KI, THPP@qTiO<sub>2</sub> and ZnTHPP@qTiO<sub>2</sub> reduced *S. aureus* viability by approximately 5 and >4 log units, respectively, whereas TCPP@qTiO<sub>2</sub> showed little activity despite efficient radical generation. None of the sensitized materials produced substantial photoinactivation of *K. pneumoniae* under the same conditions. KI markedly changed this pattern. TCPP@qTiO<sub>2</sub>, THPP@qTiO<sub>2</sub>, and ZnTHPP@qTiO<sub>2</sub> reduced *S. aureus* counts to the detection limit, while TCPP@qTiO<sub>2</sub> also achieved a >5-log reduction in *K. pneumoniae*. Hyp@qTiO<sub>2</sub> responded to KI potentiation but underwent aggregation in the bacterial assay medium and was additionally disadvantaged by irradiation outside its principal visible absorption range.

These results show that cell-free ROS generation cannot serve as a stand-alone predictor of antimicrobial performance. The biological effect depends on whether the photochemistry of the sensitizer–semiconductor system can be translated into oxidative damage at the microbial target. Surface properties, colloidal behavior, bacterial-envelope accessibility, and secondary iodide chemistry can therefore determine whether strong intrinsic photoreactivity results in effective bacterial inactivation.

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## Declarations

## Ethical Approval

Not applicable. This study involved in vitro experiments with bacterial strains and did not involve human participants or animals.

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## Availability of data and materials

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing financial or non-financial interests.

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